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Toxicity and Bioaccumulation of Endrin and Methoxychlor in Aquatic Invertebrates and Fish

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16. ABSTRACT

Stoneflies, caddis-flies, isopods, snails and bullheads were exposed to endrin or methoxychlor in a flowing-water test system for 28 days. Endrin was more toxic than methoxychlor. Behaviour changes in the caddis-fly Brachycentrus americanus and in the stonefly Pteronarcys dorsata were observed within 4 days at 0.07 and 0.15 µg/litre of endrin, respectively. The LC50 values decrease with increasing exposure times. The 28-day LC50 was less than 0.03 µg/litre for B. americanus and 0.07 µg/litre for P. dorsata. The 28-day LC50 for the bullhead Ictalurus melas was 0.10 µg/litre.

Methoxychlor, at the concentrations tested, did not affect all the species. The isopod Asellus communis was the most sensitive; behavioural changes occurred within the first 4 days. The 28-day LC50 was 0.42 µg/litre. The LC50 for the caddis-fly Hydropsyche sp. decreased from 2.9 µg/litre at 14 days to 1.3 µg/litre at 28 days. The stonefly P. dorsata and the snail Physa integra did not die at the highest concentration tested (4.2 µg/litre), although behavioural changes were seen at 2.2 µg/litre for the stonefly.

Pesticide accumulation was determined in the stoneflies and snails that survived the exposures. Stoneflies accumulated methoxychlor and endrin to concentrations 350 to 1150 times greater than the water concentrations. The snail concentration factors for methoxychlor ranged from 5000 to 8600.

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TOXICITY AND BIOACCUMULATION OF ENDRIN AND METHOXYCHLOR IN AQUATIC INVERTEBRATES AND FISH

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ABSTRACT

Stoneflies, caddis-flies, isopods, snails and bullheads were exposed to endrin or methoxychlor in a flowing-water test system for 28 days. Endrin was more toxic than methoxychlor. Behaviour changes in the caddis-fly Brachycentrus americanus *and in the stonefly* Pteronarcys dorsata *were observed within 4 days at 0·07 and 0·15 µg/litre of endrin, respectively. The* LC_{50} *values decrease with increasing exposure times. The 28-day* LC_{50} *was less than 0·03 µg/litre for* B. americanus *and 0·07 µg/litre for* P. dorsata. *The 28-day* LC_{50} *for the bullhead* Ictalurus melas *was 0·10 µg/litre.*

Methoxychlor, at the concentrations tested, did not affect all the species. The isopod Asellus communis *was the most sensitive; behavioural changes occurred within the first 4 days. The 28-day* LC_{50} *was 0·42 µg/litre. The* LC_{50} *for the caddis-fly* Hydropsyche *sp. decreased from 2·9 µg/litre at 14 days to 1·3 µg/litre at 28 days. The stonefly* P. dorsata *and the snail* Physa integra *did not die at the highest concentration tested (4·2 µg/litre), although behavioural changes were seen at 2·2 µg/litre for the stonefly.*

Pesticide accumulation was determined in the stoneflies and snails that survived the exposures. Stoneflies accumulated methoxychlor and endrin to concentrations 350 to 1150 times greater than the water concentrations. The snail concentration factors for methoxychlor ranged from 5000 to 8600.

INTRODUCTION

The toxicity of fifteen pesticides in aquatic systems has been summarised by the US Environmental Protection Agency (1976). An analysis of these reports shows that more toxicity and bioaccumulation information is available for fish than for invertebrates. The analysis also shows that a majority of the invertebrate tests only measure survival after a 4-day exposure. The lack of long-term invertebrate

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exposures is of special concern since it is well known that longer exposures result in reduced survival at lower concentrations than the short 4-day exposures. The reasons for the toxic effects at lower concentrations are not completely clear but it has been established, at least for life-cycle fish exposures, that certain life stages are more sensitive (McKim, 1977).

Assessment of long-term effects of pesticides on macroinvertebrates is limited by the lack of data and the inability to rear these animals through a complete life-cycle in the laboratory. In this study, we have attempted a compromise between the life-cycle exposure and the 4-day test by exposing the animals for 28 days. Our exposures were conducted with endrin or methoxychlor and we used survival and changes in behaviour as measures of toxic effects. In addition, the amount of pesticide accumulated by the survivors was determined. Endrin was selected because of its extreme toxicity to fish (Grant, 1976). Methoxychlor was selected because of its comparatively moderate toxicity to fish.

MATERIALS AND METHODS

Test animals

The animals used in the exposures were collected from lakes, ponds and streams in the Duluth, Minnesota, area. All animals were acclimated to the test temperature for at least a week before testing. The animals were identified using the method of Harden & Mickel (1952) for the stonefly, that of Ross (1944) for the caddis-flies, that of Williams (1972) for the isopod and those of Clampitt (1970) and Eddy & Underhill (1974) for the snail and fish, respectively.

For the endrin exposures the caddis-fly *Brachycentrus americanus* and the stonefly *Pteronarcys dorsata* were used. For a vertebrate comparison the bullhead *Ictalurus melas* was also exposed. Test animals for the methoxychlor exposures included the caddisfly *Hydropsyche* sp., the stonefly *Pteronarcys dorsata*, the isopod *Asellus communis* and the snail *Physa integra*. The invertebrates *P. dorsata* and *A. communis* were maintained before and during the exposure on a diet of birch and elm leaves collected in autumn and soaked in lakewater, *P. integra* on lettuce and leaves and *B. americanus* on adult brine shrimp. The fish were fed a combination of adult brine shrimp and trout pellets.

Exposure system

Both tests were conducted with syringe-injection, intermittent-flow exposure systems described by DeFoe (1975). The system delivered five concentrations and a control to duplicate exposure chambers. The concentrations were $0{\cdot}03 \pm 0{\cdot}01$, $0{\cdot}07 \pm 0{\cdot}02$, $0{\cdot}15 \pm 0{\cdot}02$, $0{\cdot}26 \pm 0{\cdot}05$ and $0{\cdot}60 \pm 0{\cdot}11$ µg/litre for endrin and $0{\cdot}15 \pm 0{\cdot}04$, $0{\cdot}42 \pm 0{\cdot}10$, $1{\cdot}30 \pm 0{\cdot}17$, $2{\cdot}17 \pm 0{\cdot}46$ and $4{\cdot}23 \pm 0{\cdot}84$ µg/litre for methoxychlor. The flow rate was 22 litres an hour to each chamber. Fluorescent

bulbs provided a light intensity of 380–480 lux at the water surface. The lights were automatically controlled at a photoperiod of 14 h.

Unfiltered Lake Superior water was used for all rearing and testing. Dissolved oxygen, alkalinity, pH and hardness were determined during the test; methods outlined by the American Public Health Association *et al.* (1975) were used. Dissolved oxygen was never less than 95% saturation. Other measurements, including alkalinity (42–44 mg/litre), pH (7·6–7·8) and hardness (46–48 mg/litre), were similar to those described by Biesinger & Christensen (1972) for the same water source. All exposures were carried out at 15 ± 1 °C.

Exposure procedure and monitoring

Ten animals of each species were used in each duplicate chamber. All animals (except the fish) were exposed in stainless steel screen containers that were either placed on the bottom or suspended from the side of the glass test tank. The fish were exposed in 60-litre aquaria containing 27 litres of water. During the 28-day exposure period, all test animals were observed daily for toxic or behavioural effects. The observed behavioural changes included spontaneous twisting and twirling swimming movements by the stoneflies and fish. Isopods exposed to methoxychlor were found in a non-active curled position on the bottom of the test container. When in the curled position, a gentle prodding with a glass rod would produce rapid leg movement and attempts at righting. Snail mortality was monitored by gently touching the animals' tentacles. Death was defined as a complete immobilisation and failure of the animal to respond to proddings.

Toxicant preparation and analysis

The methoxychlor and endrin test solutions were prepared in acetone from stocks containing 96·1% endrin and 99·8% methoxychlor. Because of the unknown effect of acetone, the stock solutions were adjusted so that each tank, including the control, received a nominal acetone concentration of 4·3 µl/litre.

Samples for pesticide analysis were taken once a week from each exposure tank and were analysed by gas chromatography according to procedures described by Thompson (1974). To determine the accuracy of the extraction procedures, known amounts of pesticide were added to a control-water sample before extraction. Mean percentage recovery for the water analysis was 95 ± 7 ($N = 7$) for endrin and 89 ± 12 ($N = 7$) for methoxychlor. All calculations were based on measured values corrected for recovery.

Residue analysis

At the end of the test, survivors were rinsed in control water, blotted, weighed and frozen until analysis. Two to four snails constituted a sample for the methoxychlor residue analysis ($N = 24$). The stoneflies were analysed individually ($N = 22$ for methoxychlor, $N = 17$ for endrin). Samples were extracted and analysed with gas

chromatography by using procedures described in Thompson (1974). The accuracy of the extraction and analysis was checked by adding known amounts of each pesticide to a portion of the control samples. Recovery in the residue analysis was 85% for endrin and 93% for methoxychlor.

Statistical analysis

In this study, both LC_{50} and EC_{50} values were determined using the straight-line graphical procedure described by the American Public Health Association *et al.* (1975). The term LC_{50} is used to describe the concentration which would kill 50% of the animals. The term EC_{50} is used to describe the concentration at which 50% of the animals were behaviourally unaffected.

RESULTS AND DISCUSSION

Methoxychlor

Toxicity: The isopod *Asellus communis* was the most sensitive species tested (Table 1). Ten per cent of the isopods died during the first 4 days at 1·3 μg/litre (Fig. 1). Following the first 4 days, the number of deaths increased with increased

TABLE 1

SUMMARY OF METHOXYCHLOR LC_{50} AND EC_{50} VALUES (μg/litre) FOR AQUATIC INSECTS, AN ISOPOD AND A SNAIL

Species		*Days of exposure*				
		4	7	14	21	28
Isopod						
Asellus communis	B[a]	1·78	1·45	0·89	0·64	0·42
	S[a]	—	1·75	1·15	0·75	0·42
Caddis-fly						
Hydropsyche sp.	S	—	—	2·90	1·50	1·30
Stonefly						
Pteronarcys dorsata		No deaths at 4·20 μg/litre				
Snail						
Physa integra		No deaths at 4·20 μg/litre				

[a] B = behavioural EC_{50}, S = survival LC_{50}.

exposure time. The LC_{50} decreased from 1·75 μg/litre at 7 days to 0·42 μg/litre at the termination of the experiment at 28 days. During the exposure the isopods exhibited a change in behaviour that is most simply described as lying on their side or back with random leg movement. In this position the isopod might be considered 'effectively dead' since it did not respond in a normal manner to prodding and certainly would not survive in a non-laboratory system. If immobilisation were used as the end-point, then the EC_{50} would be lower than the LC_{50} at each time interval. The percentage of test animals surviving or unaffected at various concentrations and time invervals is shown in Fig. 1.

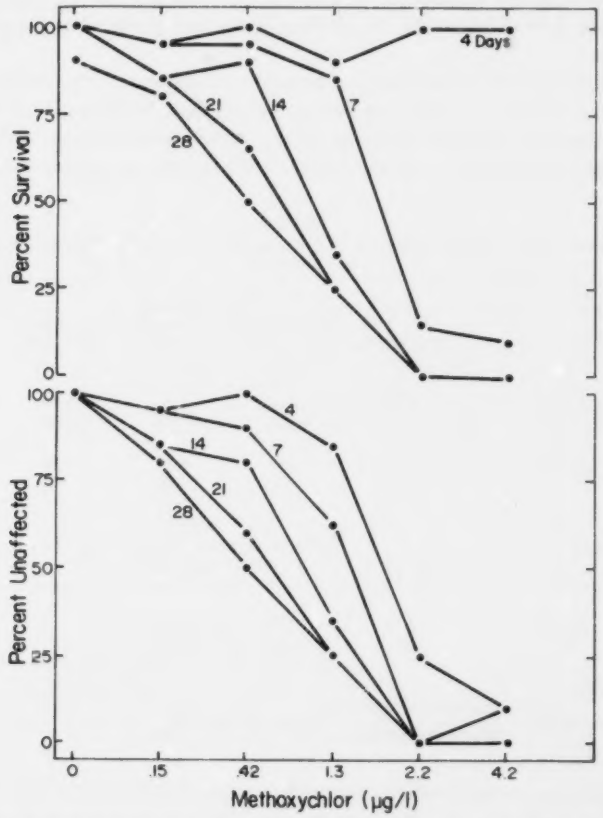

Fig. 1. Effects of methoxychlor on survival (top) and normal behaviour (bottom) of *Asellus communis* after exposure for 4 to 28 days.

Methoxychlor had little effect on survival of *Hydropsyche* sp. during the first week. During the second to the fourth weeks, toxic action was evidenced by a change in LC_{50} from 2·90 µg/litre to 1·30 µg/litre (Table 1). No stoneflies or snails died during the 28-day exposure period. Spontaneous locomotory activity of individual stoneflies increased after 2 days at the highest concentration of 4·23 µg/litre. After 14 days the number of over-active nymphs (when compared with the controls) increased at 2·17 µg/litre. No behavioural changes occurred at 1·30 µg/litre during the test period.

At 28 days LC_{50} values ranged from 0·42 µg/litre to more than 4·23 µg/litre of methoxychlor in the water (Table 1). This range is greater than that of the 28-day

LC_{50} values reported by Merna & Eisele (1973) for *Stenonema interpunctatum* (0·22 μg/litre), *Cheumatopsyche* sp. (0·49 μg/litre) and *Gammarus pseudolimnaeus* (0·29 μg/litre).

Bioaccumulation: Because of the combination of high mortality and small size, no residues were determined with isopods or caddis-flies. Methoxychlor residues in stoneflies, however, increased with increasing water concentrations and reached a maximum of 1·46 μg/g at a concentration of 4·2 μg/litre in the water (Table 2).

TABLE 2
ACCUMULATION OF ENDRIN AND METHOXYCHLOR IN STONEFLIES AND METHOXYCHLOR IN SNAILS AFTER 28-DAY EXPOSURE

Species	*Methoxychlor* *Water concentration (μg/litre ± 1 SD)*					
	0	0·15 ± 0·04	0·42 ± 0·10	1·30 ± 0·17	2·17 ± 0·46	4·23 ± 0·84
Stonefly						
Pteronarcys dorsata	0	0·17[a] (1130)[b]	0·28 (667)	0·46 (354)	0·81 (368)	1·46 (348)
Snail						
Physa integra	0	—	3·60 (8570)	6·50 (5000)	16·50 (7500)	28·2 (6710)

Species	*Endrin* *Water concentration (μg/litre ± 1 SD)*			
	0	0·03 ± 0·01	0·07 ± 0·02	0·15 ± 0·02
Stonefly				
Pteronarcys dorsata	0	0·31 (1033)	0·070 (1000)	0·091 (610)

[a] μg/g wet weight.

[b] Concentration factor = $\frac{\text{Animal concentration}}{\text{Water concentration}}$ based on wet weight.

The concentration factor for the stonefly ranged from 1130 to 348, decreasing with increasing water concentration. The snail bioaccumulated between thirteen and twenty times more methoxychlor from the same water concentrations than the stonefly. The methoxychlor concentrations in the snails was 5000 to 8570 times greater than the water concentrations (Table 2).

Endrin

Toxicity: The caddis-fly *B. americanus* was the most sensitive to endrin of the animals tested (Table 3). Toxic effects of endrin were noted after the second day at the highest concentration (0·60 μg/litre). The LC_{50} value at 4 days was 0·34 μg/litre. The LC_{50} was lower after 7 and 14 days of exposure. After 14 days the only survivors were at the lowest concentration of 0·03 μg/litre (Fig. 2) so an LC_{50} could not be calculated. Behavioural changes were also observed within the first 4 days of exposure (Fig. 2). The caddis-flies left their cases and were found lying on the bottom of the test cage, often in a curled configuration.

TABLE 3
SUMMARY OF ENDRIN LC_{50} AND EC_{50} VALUES (μg/litre) FOR AQUATIC INSECTS AND ONE FISH SPECIES

Species		*Days of exposure* 4	7	14	21	28
Caddis-fly *Brachycentrus americanus*	B[a]	0·23	0·13	0·017	—	—
	S[a]	0·34	0·21	0·044	>0·034	>0·034
Stonefly *Pteronarcys dorsata*	B	0·22	0·20	0·18	0·05	0·05
	S	—	0·28	0·20	0·13	0·07
Bullhead *Ictalurus melas*	S	0·45	0·34	0·19	—	0·10

[a] B = behavioural EC_{50}, S = survival LC_{50}.

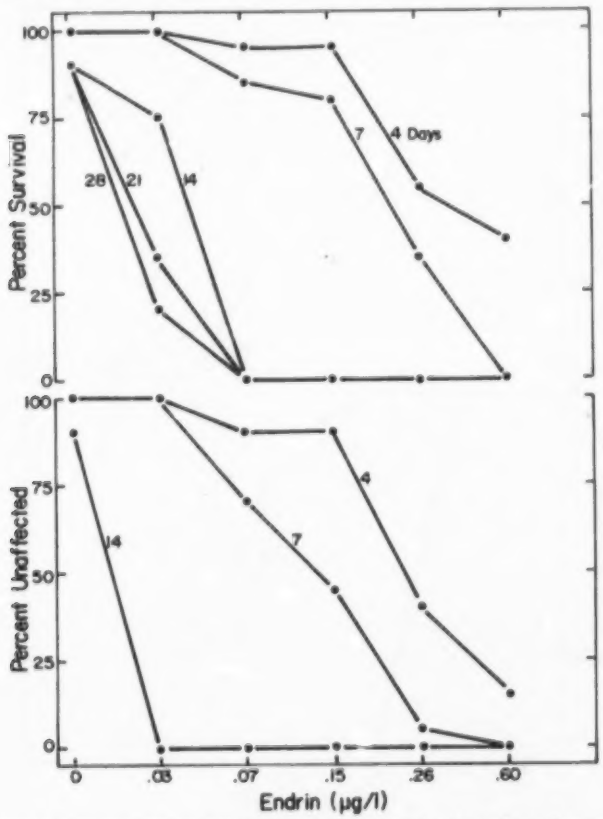

Fig. 2. Effects of endrin on survival (top) and normal behaviour (bottom) of *Brachycentrus americanus* after exposure for 4 to 28 days.

The stonefly was not as sensitive as the caddis-fly. Mortality was not high before 4 days of exposure (Fig. 3). Increased exposure time increased the mortality; the LC_{50} decreased from 0·28 at 7 days of exposure to 0·07 μg/litre at 28 days (Table 3). The behavioural changes seen with the methoxychlor exposure were also evident with endrin exposure. Percentages of test animals affected at various times and concentrations are shown in Fig. 3.

To provide a vertebrate comparison, the bullhead *Ictalurus melas* was also exposed to the same concentrations. After 28 days of exposure the bullhead is at least three times less sensitive than the caddis-fly (Table 3). The LC_{50}'s for the bullhead are similar to those for the stonefly.

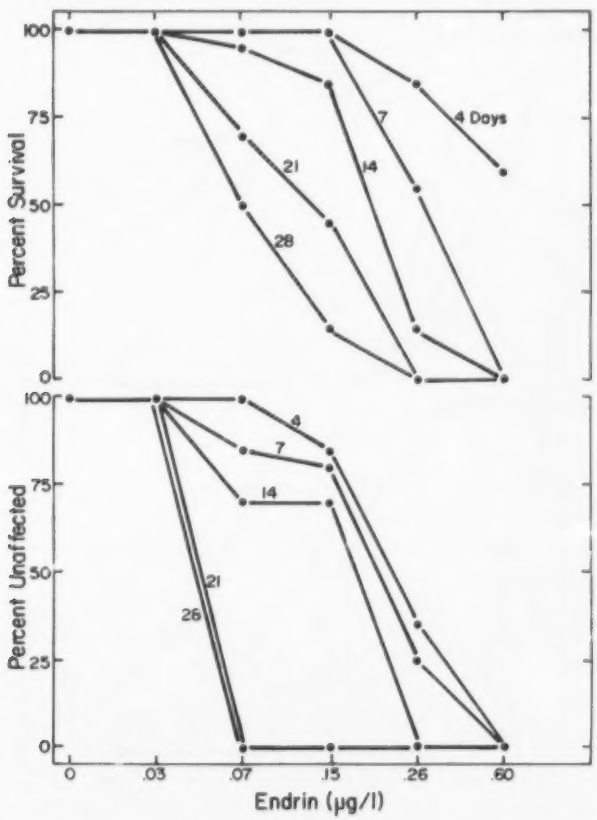

Fig. 3. Effects of endrin on survival (top) and normal behaviour (bottom) of *Pteronarcys dorsata* after exposure for 4 to 28 days.

Bioaccumulation: Stoneflies exposed to endrin for 28 days accumulated the chemical to concentrations 600 and 1000 times greater than the water concentrations (Table 2). These concentration factors are similar to those obtained for the methoxychlor exposure and may represent a 'typical' concentration factor for these types of compound for this species.

Residues were also determined for the bullhead. Samples were taken at 4 days from the 0·60 μg/litre tank and at 7 days from the 0·26 μg/litre tank. The average and standard deviation for the residues were $2{\cdot}2 \pm 0{\cdot}5$ μg/g ($N = 5$) for the 4-day exposure and $1{\cdot}6 \pm 0{\cdot}2$ μg/g ($N = 8$) for the 7-day exposure. This represents concentration factors of 3700 and 6200 for the 4- and 7-day exposures, respectively. These factors are between four and ten times higher than those found for the stonefly, even though the insect was exposed more than four times longer.

The bullhead accumulation of endrin is greater than that reported for channel catfish *Ictalurus punctatus* or bluegill *Lepomis macrochirus*. In the catfish study (Argyle *et al.*, 1973), fingerlings exposed to 0·5 μg/litre had residues of 0·2 μg/g after 5 days of exposure and 0·38 μg/g after 19 days. Continuous exposure of bluegills *Lepomis macrochirus* for 24 h at concentrations of 0·2 and 2 μg/litre resulted in accumulation factors of 250 and 150, respectively (Bennett & Day, 1970).

Toxicity comparisons

Methoxychlor and endrin affected behaviour, decreased survival and were accumulated in the test animals. The toxic effects were first seen by changes in behaviour which were followed, in most cases, by death. Behavioural effects of pesticides on aquatic macroinvertebrates have been reported by Jensen & Gaufin (1964, 1966) and by Sanders & Cope (1968). Their reported sequence of events, from hyperactivity to loss of equilibrium to tremors, convulsions and death, were observed in this study in isopods and caddis-flies at all methoxychlor concentrations. Methoxychlor at 2·2 μg/litre produced hyperactive stoneflies after 14 days of exposure but the other sequential behavioural changes were not seen and no deaths were recorded. All concentrations of endrin produced the same series of behavioural changes in caddis-flies and stoneflies.

There are only a few studies which have exposed aquatic macroinvertebrates to pesticides for longer than 4 days (Jensen & Gaufin, 1966; Merna & Eisele, 1973; Sanders, 1972). The data from those reports and the data obtained in this study show that as the time of exposure increases the toxic effects are seen at lower concentrations. But, more important than this general relationship is the difference in sensitivity among the test species. A 4-week exposure of caddis-flies *Cheumatopsyche* sp. to methoxychlor resulted in an LC_{50} of 0·42 μg/litre (Merna & Eisele, 1973). In our test, exposure of a closely related caddis-fly *Hydropsyche* sp. resulted in an LC_{50} of 1·30 μg/litre, which is about three times less sensitive. No other methoxychlor data are available to allow comparisons between the other species. However, within our test, the isopod was at least ten times more sensitive to methoxychlor than the stonefly or snail.

Similar interspecific comparisons can be made with the endrin data from this test. Jensen & Gaufin (1966) report 30-day endrin LC_{50} values of 1·2 μg/litre for *Pteronarcys californica* and 0·035 μg/litre for *Acroneuria pacifica*. In our report the LC_{50} for *Pteronarcys dorsata* was 0·07 μg/litre or about 17 times lower than the reported value for *P. californica*. In our endrin exposure, the most sensitive species was the caddis-fly *Brachycentrus americanus*. Only 20% of the larvae at the lowest concentration we tested (0·030 μg/litre) survived, while control survival was 90%. Since there are no other data for this group of insects, no direct comparisons can be made. However, our data show that extremely low concentrations (< 0·03 μg/litre) of certain pesticides are lethal to certain invertebrates.

Species sensitivity differences were shown by Flannagen *et al.* (1979) in a natural population in the Athabasca River in Canada. The population was exposed to a single 15-min pulse of 300 μg/litre of methoxychlor. As the pulse moved downstream a catastrophic drift situation was correlated with the arrival of the pesticide at the sampling stations. At a station, caddis-fly drift continued longer than mayfly or stonefly drift, suggesting that this order was affected differently than the other orders. Also within each order, individual species were drifting at different rates.

Bioaccumulation comparisons

This study shows the first laboratory bioaccumulation factors (BCF) for the stonefly *Pteronarcys dorsata*. The average BCF for methoxychlor is 573 with a range of 348 to 1130; for endrin the average is 881 with a range of 610 to 1033. Comparative interpretation of these values is difficult because of a lack of insect bioaccumulation values. Insect samples collected from the Athabasca River after a single 15-min pulse of methoxychlor at 300 μg/litre showed a maximum concentration factor of 33 (Flannagen *et al.*, 1979). This value was the result of a relatively short exposure—since the animals were exposed only as the pulse passed by—and cannot be directly compared with the results of our longer laboratory exposure. Fish bioconcentration factors for endrin range from 1640 for channel catfish *Ictalurus punctatus* (Argyle *et al.*, 1973) to 15,000 for the flagfish *Jordanella floridae* (Hermanutz, 1978).

It is evident that the fish concentrate these two pesticides more than stoneflies. The average BCF for snails exposed to methoxychlor is 6945 with a range of 5000 to 8570. Because of the lack of data, comparisons with other reported BCFs are not possible. However, in this study, the snail accumulated eight to fourteen times more methoxychlor than the stoneflies over the same exposure period.

Our results re-emphasise the importance of the relationship between the length of exposure and concentration of the test chemical. If the exposure time is sufficient, very low concentrations could cause significant reductions in the survival of some species. However, exposure for a time less than that sufficient for survival effects can affect behaviour. The hyperactive stonefly or the curled and inactive isopod could not continue its normal functions and must be classed as 'effectively dead'. The

occurrence of behavioural effects may provide another measure of toxic effects and could replace the sensitive life-stage concept (McKim, 1977) for those invertebrates whose life-cycle requirements are unknown.

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